A STUDY OF THE COMPARATIVE EFFECTS OF SOLUTIONS OF POTASSIUM, SODIUM AND CALCIUM CHLORIDES ON SKELETAL AND HEART MUSCLE.

DISSERTATION

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

ACAD. MED. BIBL.

BY A. E. GUENTHER.

BALTIMORE, MD.

JUNE, 1905.

Reprinted from the American Journal of Physiology—Vol. XIV, July 1, 1905. No. I.

Reprinted from the American Journal of Physiology.
Vol. XIV. — July 1, 1905 — No. I.

A STUDY OF THE COMPARATIVE EFFECTS OF SOLUTIONS OF POTASSIUM, SODIUM, AND CALCIUM CHLORIDES ON SKELETAL AND HEART MUSCLE.

By A. E. GUENTHER.

[*From the Physiological Laboratory of the Johns Hopkins University, Baltimore, Md.*]

THE very numerous facts which have been published since 1875, when Merunowicz[1] showed the relation existing between the inorganic constituents of the frog's serum and the activity of the heart, indicate with a high degree of probability the essential importance of potassium, calcium, and sodium salts in the maintenance of the normal condition of muscle tissue. These salts, in order that they may be most efficacious, must exist in definite proportions in the solution surrounding the muscle, and in such amounts as to form a medium isosmotic with the tissue fluids. For the terrapin's heart, as an example, the proportions must approximate those in a Ringer solution of NaCl 0.7 per cent, $CaCl_2$ 0.026 per cent, and KCl 0.03 per cent.[2] The percentages of these salts may vary within limits depending upon unknown differences in the kind of muscle[3] and upon the condition in which a muscle may chance to be at a given time.[4]

In 1899 Loeb[5] called attention to the fact that the production of the specific effects of the salts in question is intimately connected with their being in a state of dissociation. Since then it has been generally agreed that the physiological changes produced are ionic effects. It is highly probable that anions,[6] as well as kations, influ-

[1] Merunowicz: Arbeiten aus der physiologischen Anstalt zu Leipzig, 1875, x, p. 132.

[2] Greene: This journal, 1898, ii, p. 82.

[3] Row: Journal of physiology, 1903, xxix, p. 440.

[4] Ringer: Journal of physiology, 1887, viii, pp. 20, 288; Martin: This journal, 1904, xi, p. 103.

[5] Loeb: Ueber Ionen welche rhythmische Zuckungen der Skelettmuskeln bevorrufen, Festschrift für A. Fick, Braunschweig, 1899.

[6] Loeb: This journal, 1900, iii, p. 327.

ence the reactions of an immersed muscle, but the importance of the kations is both greater and more immediate. The method of their action is by no means clear. Loeb[1] considers the ions as forming definite chemical combinations with the living matter of the muscle so that there may be sodium-, calcium-, or potassium-proteid compounds. The proportion of these compounds formed depends upon the relative number of sodium, calcium, and potassium ions which leave the medium surrounding the muscle and diffuse into the tissue. If any one ion increases in numbers, it supplants a certain number of the ions of other ion-proteid compounds until the relative proportions of the latter are the same as those of the uncombined ions.

The impression given by Loeb's published statements on this point is that the substance of the muscle fibre is freely permeable to ions, but that the sarcolemma forms a more or less semipermeable membrane. In contrast to this stands the more definitely expressed opinion of Overton[2] that there is little if any diffusion into an inactive muscle. The latter is regarded as a complex system of structures of limited permeability surrounded by sarcolemma and perimysium of entirely different osmotic properties. The sarcolemma and connective tissue envelopes offer no resistance to the diffusion of most crystalloids in solution, but the muscle fibres themselves as long as they remain normal are impermeable, or nearly so, to the majority of inorganic substances. During the process of contraction, however, the permeability is altered so that diffusion takes place to an extent sufficient to produce physiological effects. Overton[3] states that when a muscle is immersed in a solution of electrolytes under such conditions that physiological effects are produced the concomitant diffusion does not follow the ordinary laws of diffusion, so that it is necessary to assume an active participation of the living muscle.

There is a general consensus of opinion that potassium salts in a bathing medium produce, or tend to produce, an inhibition of vital processes if they are present in sufficient quantities, but considerable differences of opinion exist with regard to the exact rôle played by the kations calcium and sodium. The investigation of the part they play has been prosecuted most vigorously in connection with contrac-

[1] Loeb: This journal, 1900, iii, p. 327.

[2] Overton: Archiv für die gesammte Physiologie, 1902, xcii, p. 115.

[3] Overton: Archiv für die gesammte Physiologie, 1904, cv, p. 207.

tion phenomena. In 1893 there appeared a research by Howell and Cooke [1] in which the authors undertook an investigation of the relative nutritive powers of serum albumin and serum globulin in the maintenance of the heart in activity. Their work led them to a special study of the sodium-calcium-potassium mixture on account of its remarkable power of sustaining the rhythmicity of the heart. A series of papers brought out under Howell's initiative culminated in his well-known theory of the relation of sodium, calcium, and potassium salts to the production of the heart beat. This theory [2] is the logical outcome of the fundamental work begun by Ringer.[3] It lays special emphasis upon the importance of calcium.

Seemingly opposed to this theory is the one of Loeb, which, based upon Biedermann's observation made in 1881 that a frog's sartorius will twitch in an alkaline solution of sodium salts, has been used to interpret the experimental results obtained from a great variety of rhythmically contractile tissues. Loeb's views [4] are of interest here only in so far as stress is laid upon the importance of the sodium ion in the production of rhythmicity. In 1900 Lingle [5] attempted to apply Loeb's views to the phenomena of the beating heart. He differentiated between agents which start a series of beats and those which maintain the series when once started, placing sodium among the former and calcium among the latter. The rhythmical contractions of the heart in 0.7 per cent saline are regarded as homologous to the twitches of striated muscle in the same solution. It is indeed desirable to establish a parallel in the reactions of the two tissues which, though alike in some respects, are nevertheless unlike in others. The inhibitory effect which calcium in physiological amounts exerts upon the twitches of skeletal muscle immersed in saline becomes an augmentor effect when this ion is applied to heart strips beating in saline.

A rapidly growing literature has made it strikingly evident that the reactions of a great variety of tissues to the same electrolytes differ markedly. In illustration of this may be cited the fact that potassium salts are less injurious to many plant cells than are the

[1] Howell and Cooke: Journal of physiology, 1893, xiv, p. 198.

[2] Howell: This journal, 1902, vi, p. 181.

[3] Ringer: Journal of physiology, 1883, iv, pp. 29, 370; 1884, v, p. 352; 1885, vi, p. 154; 1886, vii, p. 291; 1887, viii, pp. 20–288.

[4] Loeb: Ueber Ionen welche rhythmische Zuckungen der Skelettmuskeln bevorrufen, Festschrift für A. Fick, Braunschweig, 1899.

[5] Lingle: This journal, 1900, iv, p. 270.

corresponding sodium salts; the reverse being true for muscle fibres.[1] Lillie[2] has shown that pure sodium chloride solutions produce rapid deterioration of the cilia of Arenicola larvæ, while Maxwell[3] has found it comparatively innocuous to the cilia lining the frog's œsophagus. Pure distilled water is generally agreed to be harmless to fresh-water organisms, but Bullot[4] has demonstrated that this is not true for the fresh-water Gammarus. Many other examples might be given. Cardiac and skeletal muscle present two important points of difference in their power of response to excitation. The former is characterized by a relatively long refractory period and by the fact that its contractions obey the "all or none" principle. The possibility is apparent that these tissues may likewise differ in their reactions to salts in solution. For this reason it has seemed desirable that a detailed study should be undertaken of the similarities and dissimilarities of the effects of sodium, calcium, and potassium chloride upon heart and skeletal muscle.

This work was done at the suggestion and under the direction of Dr. Howell, to whom the author is greatly indebted for ever constant interest and guidance.

The apparatus varied with the requirements of the experiments, but was always of the simplest description. The distilled water and the salts were prepared after the customary manner of this laboratory, as described by Greene.[5] The amount of the solutions used was twenty-five cubic centimetres at a time. In the majority of the tests the sartorius of the frog and heart strips from the ventricle of the terrapin were the tissues used. Experiments carried out on other muscles of the frog show that different muscles exhibit minor variations. Warm weather also modifies the results.[6]

Tone Changes.

Pure isotonic solutions. — When the sartorius muscle which has been carefully removed from the body of a frog is suddenly surrounded by a 1 per cent solution of potassium chloride (approximately isotonic with the body fluids), it exhibits a moderately rapid contraction

[1] Overton: Archiv für die gesammte Physiologie, 1904, cv, p. 207.
[2] Lillie: This journal, 1901, v, p. 56.
[3] Maxwell: This journal, 1905, xiii, p. 154.
[4] Bullot: University of California publications, Physiology, 1904, i, p. 199.
[5] Greene: This journal, 1898, ii, p. 82.
[6] Martin: This journal, 1904, xi, p. 103.

followed by a somewhat slower relaxation, both changes being readily followed with the eye, and one or both may be either regularly progressive or made up of a series of rapid twitches. The relaxation may not be complete. It may be marked by a small contraction remainder which slowly disappears. Or if the muscle happens to be in condition of slight contraction brought on by handling or otherwise, it may relax to a length greater than that before immersion. In any case, if allowed to remain in the solution, it continues in an extended condition, showing, however, a second very slight but constant variation in its length. Experiments in which the muscle is so arranged as to record this variation upon a slowly revolving drum give evidence that the initial potassium contraction is followed by an almost imperceptible lengthening during the first quarter of an hour, after which the muscle grows shorter.

This second contraction continues for about twenty-four hours, when it reaches a maximum. The immersed sartorius now relaxes at a somewhat more rapid rate than the previous shortening, until the writing-point has sunk far below the level of the starting-point. Extreme relaxation is reached from the fourth to the sixth day after immersion, when in nearly all cases putrefaction is well advanced. The details of a typical experiment are recorded in column 2 of Table I, in which, however, the changes in length due to the first potassium contraction are omitted.

The relatively rapid changes in length resulting from the first contact of the muscle with the solution, and which may be designated the "initial potassium contraction" are omitted for the present and will be discussed separately later on. A 2 per cent solution of potassium chloride gives qualitatively the same results as a 1 per cent solution. Miss Moore[1] has described a somewhat similar behavior of the gastrocnemius. When placed in strong solutions, an immediate strong contraction with occasional twitching occurs. This is followed by a slight relaxation, which sometimes amounts to one-half of the original contraction. Contraction then begins again and continues until rigor is complete. After rigor complete relaxation again occurs.

These changes in length as given in Table I follow in a general way the changes in weight which the muscle exhibits. These have been determined by Overton,[2] who states that the muscle may after twenty to twenty-four hours attain double the original weight, after which it

[1] Moore: This journal, 1902, vii, p. 1.

[2] Overton: Archiv für die gesammte Physiologie, 1904, cv, p. 207.

loses again until it is reduced to one and one-half times the original weight. He states that during this time the muscle shows no changes in length, but remains in an extended condition. Since the changes

TABLE I.

Changes in the length of the sartorius muscle of the frog in isotonic solutions. Magnification 3 ×. The plus and negative signs refer to positions above and below the level of the starting-point respectively.

Time.	KCl 1%. Second contraction.	NaCl 0.7%.	$CaCl_2$ 1%
	mm.	mm.	mm.
Jan. 16, 10.00 A. M.	0.00	0.00	0.00
" " 10.30 A. M.	−1.25	−2.25	
" " 2.00 P. M.	0.00	−5.25	+40.50
" " 4.00 P. M.	+1.50	−6.00	+49.50
" 17, 9.30 A. M.	+10.50	−7.50	+29.50
" " 11.00 A. M.	+18.00	−8.00	
" " 12.00 M.	+19.00	−8.50	
" " 2.00 P. M.	+4.50	−9.25	+27.00
" " 3.00 P. M.	−2.25	−9.50	
" " 5.00 P. M.	−4.25	−10.00	+25.50
" 18, 10.00 A. M.	−6.50	−13.75	+24.25
" " 5.00 P. M.	−10.00	−16.00	+23.50
" 19, 10.00 A. M.	−12.25	−17.75	+20.75
" 20, 9.00 A. M.	−17.00	−22.50	+15.50
" 21, 10.00 A. M.	−23.00	−26.75	−6.00
" 22, 3.00 P. M.	−29.00	−33.00	−6.00
" 23, 9.00 A. M.	−29.00	−33.00	

shown in Table I might possibly be due to the increased weight of the sartorius disturbing the balance of the writing-lever, a number of experiments were made in which a weight equal to that of the muscle was added to the latter. No change in the position of the writing-point occurred. It required, in fact, a load more than six times that of the muscle to overcome to an appreciable extent the friction of the recording mechanism. Either Overton's frogs differ, in this respect, from

those used here or he overlooked these small changes, which roughly amount to only one-tenth of the muscle's length.

When immersed in a 0.7 per cent sodium chloride solution, the sartorius manifests a loss of tone which reaches its maximum extent, as in the case with potassium chloride, during the fourth to the sixth day. This is shown in the third column of Table I. Usually a rapid initial fall is followed by a more gradual one, which in turn becomes accelerated when putrefaction is well under way. The fall in tone is rarely absent. Out of seventy-six tracings taken at random it failed to appear in but one case, and in all of these the characteristic sodium twitches were present. It has been determined by Overton and Loeb that there is a slight gain of weight by the muscle immersed in an isotonic sodium chloride solution. According to Loeb [1] the gain varies from 6 to 8 per cent of the original weight during eighteen hours' immersion. The fact that the shortening of the muscle is not dependent upon changes in weight, which is evident in the case of potassium chloride, is more marked in sodium chloride, where a loss in tone accompanies a gain in weight. It is also shown by the behavior of the sartorius in calcium chloride. Solutions of the latter, isotonic with 0.7 per cent sodium chloride, produce during the first twelve hours a great increase in tone which disappears slowly. This is shown in the fourth column of Table I. According to Overton [2] there occur during the first fifty-five hours of immersion in a 1 per cent calcium chloride solution a slight loss in weight and then a gain.

In the investigation of the functional alterations taking place within the muscle, changes in length, or tone changes as they are here called, form a far more delicate indicator of such alterations than do changes in weight, but in turn are less delicate than the changes which can be observed to take place in the processes of contraction and relaxation caused by an electrical stimulus. If, for example, a sartorius muscle is immersed in a 1 per cent solution of potassium chloride, certain alterations take place during the first ten to twelve minutes. Periodical stimulation of the muscle reveals a rapidly declining power of the muscle to contract. If the tone changes only are observed, there may be witnessed the single preliminary potassium contraction or potassium twitches followed by a

[1] Loeb: Archiv für die gesammte Physiologie, 1899, lxxv, p. 303.

[2] Overton: Archiv für die gesammte Physiologie, 1904, cv, p. 207.

loss of tone, while changes of weight as stated by Overton cannot be demonstrated with certainty during this period.

The experimental results upon the tone of the sartorius obtained with pure solutions of sodium, calcium, and potassium chloride isotonic with the frog's tissue fluids, show that sodium chloride permits a relaxation of the muscle which merges gradually into the final lengthening due to the disorganization of the tissue. Calcium chloride produces a slow shortening, which persists until the final disorganization, when the tissue becomes stretched under the influence of the weight which it supports. Potassium chloride produces a moderately rapid shortening and relaxation. The relaxation merges into a slower lengthening, which in turn is not readily separable from the final lengthening due to the internal disintegration of the structure of the muscle. The fact that the sartorius exhibits a rapid contraction and relaxation followed by a slower shortening and lengthening may possibly be due to a mixture of two different contractile elements which, either in Grützner's[1] or in Bottazzi's[2] sense, enter into the formation of the sartorius of the frog. It is conceivable that the two elements may differ in their response to solutions of potassium chloride in such a way that the contraction and relaxation of one take place long before the other.

The effects of pure solutions of sodium chloride and calcium chloride on terrapin's heart and on frog's sartorius are quite similar as regards tone changes. In both muscles sodium chloride produces a loss and calcium chloride a slow increase in tone. The shortening produced by calcium in the heart is more vigorous than that shown by skeletal muscle, coming on more quickly, but being of shorter duration. In both relaxation occurs sooner or later. In one instance the sartorius began to relax six hours after immersion, while the heart had already begun to relax at the expiration of the first hour. But towards potassium solutions they present a marked disagreement. Heart strips taken from the ventricle of the terrapin or frog and placed in a 1 per cent solution show no stimulation effects, but manifest only a progressive loss of tone. This forms one of the most striking differences in the reactions of the two tissues in question. However, it should be stated at this point that a reference to the literature does not bear out this point very satisfactorily. Stiles[3] has

[1] Grützner: Recueil zoologique suisse, 1884, i, No. 4. Reference taken from Biedermann's Electrophysiologie, i, p. 111. Translated by Welby.

[2] Bottazzi: Journal of physiology, 1897, xxi, p. 1.

[3] Stiles: This journal, 1903, viii, p. 271.

shown that for stomach tissue potassium chloride has a relaxing effect as long as the percentage is below 0.15, — higher doses causing a tonic contraction. Martin[1] corroborates these results for the terrapin's heart, but places the amount necessary at 0.23 per cent. "About 0.23 per cent potassium chloride appears to be the dose at which variations in tone do not occur in either direction." Greene[2] states that 1 per cent potassium chloride applied to heart strips beating previously in dilute serum resulted in one or two spasmodic contractions and then quiescence in a condition of tone. Lingle[3] has reported that a solution of potassium chloride, equimolecular with 0.7 per cent sodium chloride, always causes a tonic shortening in strips that are not beating. The author's results are not opposed to those stated above if the assumption is made that Martin's, Greene's, and Lingle's tests were not carried out on perfectly fresh strips. Fresh strips placed directly into a 1 per cent solution of potassium chloride always gave relaxation. One-half of the number of strips placed in 0.7 per cent saline for one, two, three, and four hours gave single beats upon transferal to potassium chloride. Previous immersion in Ringer for two to three hours gave rise to small contractions in the strip on subsequent treatment with potassium. Subjection to Ringer for two hours followed by a short bath in 8 per cent cane-sugar gave rise to a slow contraction and relaxation of the strip when immersed into 1 per cent potassium chloride, and these changes were of greater amplitude than when Ringer alone was used. Frequent stimulation of the strip with break-induced currents, while being subjected to Ringer solution for one-half hour was followed by a good contraction on subsequently exposing to potassium. These results were established in twenty experiments on strips taken from the ventricle of the common slider terrapin, Pseudemys rugosa, during the months of March and April. They can be accounted for by the assumption that the shortening produced by potassium is dependent upon the presence of calcium in the tissue in a particular form. If calcium is supplied by bathing the muscle in Ringer or by placing the muscle under conditions that permit the spontaneous conversion of calcium from an ineffective to an effective form, as has been supposed by Martin,[4] then the strip becomes capable of responding to the potassium solution.

[1] Martin: This journal, 1904, xi, p. 103.
[2] Greene: This journal, 1898, ii, p. 82.
[3] Lingle: This journal, 1900, iv, p. 265.
[4] Martin: This journal, 1904, xi, p. 103.

Combinations of two isotonic solutions. — The loss in tone of the sartorius which takes place when immersed in a 0.7 per cent saline solution is very slightly if at all influenced by small additions of potassium chloride. In this respect there is a point of difference between skeletal and cardiac muscle. Greene states that terrapin heart strips in 0.7 per cent sodium chloride plus 0.03 per cent potassium chloride almost always show excessive loss of tone. If the potassium is added to the sodium as a 1 per cent solution, then the mixture remains isosmotic to the tissue fluids, but the solutions become mutually diluted. If, then, while the percentage of potassium becomes greater and the percentage of sodium becomes less, the effects of these combinations on the sartorius muscle are noted, it will be observed that among the first effects of the potassium is the suppression of the characteristic sodium twitches. With a further increase of the potassium chloride a phenomenon characteristic of the potassium begins to appear, namely, the initial potassium contraction. Small at first, this potassium contraction grows as the potassium content of the mixtures is made greater. A fresh vigorous sartorius first washed in saline may respond with a minimal potassium contraction if submitted to a solution consisting of 0.7 per cent saline plus 0.04 per cent potassium chloride, although sudden immersion into saline alone produces no effect. By first immersing into saline the error which might arise as the result of closing the demarcation current is avoided.

The minimal amount of potassium necessary in a mixture of 0.7 per cent sodium chloride plus 1 per cent potassium chloride in order to produce the second shortening which the sartorius may exhibit is not easily determined. Three muscles in solutions containing less than 0.04 per cent potassium, after a preliminary fall, began to shorten at intervals varying from six to twenty-four hours after immersion; but six others showed only a loss in tone. All of twenty-five muscles placed in solutions containing more than 0.04 per cent potassium gave rise in tone.

If in a similar manner there are added gradually increasing amounts of a 1 per cent solution of calcium chloride to 0.7 per cent saline, the loss of tone in the sodium chloride is replaced by an increase in tone due to the calcium. The latter in minute doses is not so efficient in causing a shortening of the muscle as is potassium. Beginning with an amount as low as 0.05 per cent in saline, the sartorius always showed some contraction, although the variations in the extent of the short-

ening were considerable. A short preliminary fall was often observed, arising from the fact that the latent period of the calcium effect is long, and during this time the muscle lengthens under the influence of the attached load. Calcium present in saline in amounts less than 0.01 per cent always permitted the muscle to relax. Like potassium, but even more readily, it inhibits the sodium twitches.

A comparison of the effects of combinations of the chlorides of sodium and calcium on heart strips and skeletal muscle shows that the tone changes produced are in general much alike in the two tissues. Calcium, as soon as the amount exceeds that in a Ringer mixture, antagonizes the relaxation which pure saline permits. When it forms as much as 0.05 per cent of the mixture, it gives rise to a slow shortening of the muscle. Heart strips respond much more quickly to calcium than do skeletal muscles.

Mixtures of sodium chloride plus increasing doses of potassium lead only to progressive elongation in terrapin heart strips as long as they remain in a fresh condition, while such solutions will produce the characteristic potassium contraction in the animal's skeletal muscles. The rapidity with which potassium produces a contraction in skeletal muscle stands in contrast to the slow shortening due to calcium.

Combinations of the three solutions. — In studying the effects of mixtures of the three solutions — sodium, calcium, and potassium chloride — in varying proportions on the sartorius of the frog, it is convenient to begin with a Ringer solution in which the amounts exist in physiological percentages, or nearly so. The Ringer mixture permits an elongation of the muscle under the influence of the extending weight very much as in simple saline, but the elongation is, perhaps, not so great. Variations in the state of tone are readily produced by proper changes in the percentages of the three constituents. Observing the precaution that the mixture as a whole remain approximately isotonic with the tissue fluids, it may be stated that a relative increase of the calcium over the other ions produces an increase in tone. That a relative increase of potassium leads to diminished tone unless the preponderance is excessive. An increase in the relative proportion of the sodium ions leads to diminished tone as in pure saline. The effects upon ventricular heart strips from the terrapin resemble precisely as regards tone those of the frog's sartorius except in the case where potassium is present in large amounts. The preliminary potassium contraction does not take place, and as

long as the ventricular strip remains fresh only diminished tone is observed.

The effects after previous treatment. — If the actions of the salts in question are due to the diffusion of ions into and out of the muscle, it is to be expected that preliminary treatment of the muscle so as to change its ion content or perhaps change only the surface layer would affect the results obtained from subsequent treatment with the chlorides of sodium, calcium, or potassium. With this possibility in view the sartorius was given baths in 7.7 per cent cane-sugar solutions for varying lengths of time, after which followed immersions into isotonic solutions of the three chlorides, singly or in combination. The data derived from more than one hundred and fifty experiments bearing on this point may be summarized as follows: A solution of cane-sugar applied to the sartorius of the frog produced in the majority of cases no changes in tone for many hours. Eventually an increase in tone was obtained. Immersion into 0.7 per cent saline for from one to five hours altered the muscle so that the application of cane-sugar gave an immediate increase in tone in thirty-eight out of forty-three muscles. The addition of calcium chloride in physiological amounts to the sodium chloride rendered an immersed muscle more resistant to subsequent application of cane-sugar. The shortening produced by the sugar was to some extent at least readily reversed by plunging the muscle into pure solutions of sodium, calcium, or potassium. The subsequent behavior of the muscles if allowed to remain in these solutions was much as if the preliminary bath in sugar had not been given. Sodium chloride caused continuous relaxation; calcium chloride a more vigorous contraction and potassium chloride, also in time produced a slow contraction and relaxation. Brief preliminary treatment with sugar did not destroy the first potassium contraction, but longer exposure prevented its occurrence.

If after an hour's bath in sugar the sartorius is transferred to a mixture of cane-sugar and varying amounts of sodium chloride, the changes in tone, if any at all are produced, are small and in the majority of cases in the direction of relaxation. Similarly, transferal after an hour's sojourn in 8 per cent cane-sugar to mixtures of cane-sugar plus calcium in varying amounts causes a shortening of the muscle when the calcium is present in sufficient amount to produce any effect at all. If small amounts of potassium chloride, *i. e.*, physiological doses or less, are added to the sugar solution, they apparently have no effect on the tone changes. Comparatively large

amounts in sugar allow a preliminary relaxation. The tone changes produced by sodium or potassium under these conditions are almost insignificant when compared with those produced by calcium.

The results of the experiments taken altogether seem to indicate that approximately isotonic solutions of cane-sugar produce no tone changes until after long immersion or when there is reason to suppose that the muscle has begun to undergo deterioration. The sugar in its action produces a stage during which the sartorius is more sensitive in its reactions to sodium, calcium, and potassium chloride, succeeded by a later stage during which this sensitiveness is so reduced that it falls below normal.

A comparison of the effects produced by a sugar solution on the frog's sartorius with those produced in terrapin heart strips shows in general much similarity. Heart strips show a loss in tone for an hour or less followed by an increase, while the sartorius may elongate when first immersed, but the development of tone is readily produced. If calcium chloride is added to the sugar solution after the heart strip has been immersed in it for some time, it shows a tendency to go into excessive tone. The sartorius under like conditions also develops tone. If potassium chloride is added in small quantities, it either exerts no effect or produces an increased tone in terrapin heart strips. In the sartorius there is also the production of tone, although it may be of much slower development. In both ventricular strip and sartorius the increased tone due to the immersion in sugar is reversed by applying 0.7 per cent saline.

The Potassium Contraction.

Since the discovery by Bernard and Grandeau [1] that potassium salts in suitable strengths call forth contractions of skeletal muscle, this fact has been corroborated by many observers. Among these may be mentioned Zenneck,[2] Grützner,[3] Zoethout,[4] and Overton.[5] Grützner, finding a 4 per cent solution ineffective when applied to a motor nerve, got a prolonged contraction by applying the same solution to a curarized gastrocnemius. Zoethout describes briefly

[1] Bernard and Grandeau: Centralblatt für die medicinischen Wissenschaften, 1864, p. 183.

[2] Zenneck: Archiv für die gesammte Physiologie, 1899, lxxvi, p. 21.

[3] Grützner: Archiv für die gesammte Physiologie, 1893, liii, p. 83.

[4] Zoethout: This journal, 1902, vii, p. 199.

[5] Overton: Archiv für die gesammte Physiologie, 1904, cv, p. 207.

a series of contractions obtained by alternately applying potassium and calcium chloride to a gastrocnemius muscle, and calls attention to the antagonistic effects produced by the potassium and calcium respectively. Overton records the changes in the weight of a frog's sartorius when subjected to solutions of potassium chloride. No changes in weight during the first ten to twelve minutes could be determined with certainty.

The number of contractions evoked by immersions into potassium chloride is a small one. Alternation of potassium chloride with

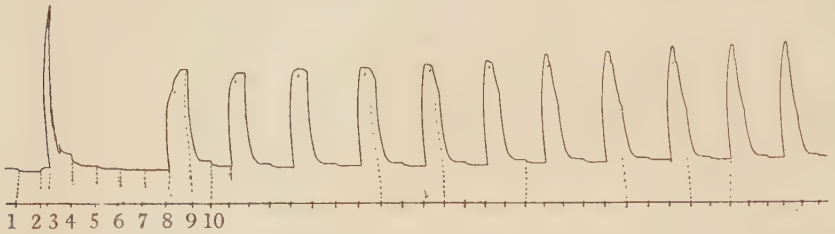


FIGURE 1. — Portion of a curve showing contractions of the sartorius muscle of a small bull-frog obtained by immersing into KCl solution. Temperature, 25°. At 1, immersion into NaCl; solution removed at 2; 3, immersion into KCl; 4, into NaCl; 5, into KCl; 6, into NaCl; 7, in $CaCl_2$. One minute each, with intervals of same duration. Solutions used were 0.7 per cent NaCl, 1 per cent KCl, and 1 per cent $CaCl_2$. The small dot near the top of each contraction indicates removal of K solution.

sodium chloride increases the number; an alternation of potassium chloride with calcium chloride gives a still greater number of contractions, and the application of the three chlorides in rotation gives the best results of all. This procedure when carried out on the sartorius of the frog gives a series of contractions like that produced by periodic electrical stimulation. The series shows sometimes one or more preliminary contractions of diminishing height analogous to the "introductory contractions," then a series of increasing height comparable to the "staircase," and finally a series of diminishing height as in "fatigue." A portion of such a curve is shown in Fig. 1, which was obtained from the sartorius of a small bull-frog. The solutions used were 1 per cent potassium chloride, 0.7 per cent sodium chloride, and 1 per cent calcium chloride, into each of which the muscle was immersed one minute at a time with intervening rests of the same duration when the muscle hung suspended in the air. At 1 the muscle was immersed in sodium chloride, which was removed at 2. At 3 the immersion was into potassium chloride. A rapid contraction followed, marked by irregular twitches on the descending limb.

At 4 the solution was replaced by sodium chloride. At 5 the muscle was again placed in potassium chloride, at 6 in sodium chloride, and at 7 in calcium chloride, and then once more, at 8, in potassium chloride. The contraction beginning with 8, and the remaining members of the series were obtained by applying potassium, sodium, and calcium in rotation.

There is, therefore, a great difference noticeable in the character of the first contraction and all that follow, — for in the latter the movement is slower and smoothly progressive; *i. e.*, the myogram shows no irregular twitches. This difference is a constant one. When the temperature is comparatively low, the muscle a vigorous one, and the subjection to the potassium chloride solution of brief duration, the muscle may respond several times to the application of the potassium, but usually there is but a single response, as shown in the accompanying curve. Whether left in the solution or withdrawn so as to hang in the air, the sartorius relaxes completely or nearly so. The behavior of the muscle when left in the solution for several days has already been described. The irritability disappears within a few minutes so that the muscle responds to neither mechanical nor electrical stimulation. Treatment with a bath of either sodium or calcium chloride restores the ability of the muscle to respond to potassium. But the series of contractions produced by an alternation of potassium and sodium differs decidedly from that produced by an alternation of potassium and calcium. The former is of shorter duration, the individual contractions of rapidly lessening amplitude, and complete relaxation to the base-line follows each shortening. In the latter the series is a longer one, but the muscle relaxes incompletely after each contraction, so that the lever rises as in contracture until the individual contractions become very small. An alternation in a similar manner of calcium chloride with sodium chloride produces no visible changes in the muscle. A series of contractions of maximum duration can be obtained only by the employment of all three solutions, — potassium, sodium, and calcium chlorides. Whether the calcium and sodium are applied separately or at once in a mixed solution matters little, except that it is better to use a mixture of 0.7 per cent sodium chloride with little calcium than a mixture of 1 per cent calcium chloride plus a small amount of sodium. The alternation of potassium chloride and a Ringer mixture also gives a good series.

As has been stated before, of the series so obtained the first contraction always (and sometimes the first two or three) differs in

character from the remainder. It possesses by far the shortest latent period which in fifteen determinations averaged 0.26 second —the extreme values varying from 0.045 second to 0.54 second. In contrast to this the latent period of the remainder of the series, as shown by seventy-one determinations made on eight muscles, equalled 0.8574 second on an average. The extreme individual values were 0.546 and 1.092 seconds. The method of obtaining the time relations of the contractions produced by the potassium chloride was the following: A drum driven at a uniform speed of five and one-half millimetres per second by an electric motor was marked by vertical lines so placed that the distance between them represented intervals of ten seconds each. The lower end of a sartorius muscle was attached to an L-shaped glass rod, while the upper end was attached to one end of a recording lever. As the drum revolved, the writing-point passed in succession the vertical lines on the drum, and at the moment of crossing a beaker holding about twenty-five cubic centimetres potassium chloride or other solution to be tested was quickly brought up so as to surround the muscle. The figures given for the latent period are, therefore, too large, as no correction was made for the reaction time, for the time required to bring the solution up around the muscle, or for variations in the size of the muscle. But they serve the purpose intended, giving qualitatively the changes produced in the reactions of the muscle under the influence of the three electrolytes.

The rapidity of shortening of the first contraction is strikingly greater than that of the others, reaching a maximum in 6.08 seconds when relaxation sets in, even though the muscle remains immersed in the potassium chloride. Usually the solutions tested were allowed to act upon the muscle ten seconds at a time, with intervening pauses of like duration during which the muscle hung in the air. Under such conditions the layer of fluid clinging to the surface of the muscle made the immersion equivalent to one twice as long. And, as a matter of fact, it was found that the exact time of removal of a solution could be varied through a considerable range without affecting the result, but the time of application had to be exact.

The time required by a muscle to reach its maximum of shortening when exposed to a solution of potassium chloride depends upon the immediately preceding treatment to which the muscle has been subjected. In a series obtained by alternately immersing the muscle into 1 per cent potassium chloride for twenty seconds and a Ringer solu-

tion for sixty seconds, it was found that the average time of shortening after the first contraction equalled 7.755 seconds. This average expresses the results of twenty determinations made on four muscles. If in place of alternating potassium chloride and Ringer, potassium, calcium, and sodium — or potassium, sodium, calcium, and sodium — are alternated within the same time limits, then the period of shortening grows to more than twenty seconds. This is the average of forty-eight determinations made on two muscles.

The first relaxation of a potassium contraction series is apparently uninfluenced by immersion in Ringer, sodium, or calcium. It runs its course, reaching the base-line in 50 per cent of the observations in about thirty seconds. In the remaining 50 per cent a small contraction remainder was still present at the end of thirty seconds, which although gradually diminishing continued for more than sixty seconds. All relaxations after the first are noticeably influenced by immersing the muscle into 0.7 per cent saline, 1 per cent calcium chloride, or 8 per cent cane-sugar. These agents all produce an augmentation of the rate of lengthening. In their effectiveness in producing this result they stand in the following order: sodium, calcium, and sugar. This may be judged by the completeness of the relaxation which results in a given time. Thus it was found that in sodium chloride the muscle relaxed 91.27 per cent of the height of contraction in ten seconds; 97.11 per cent in twenty seconds. When sodium chloride was replaced by 1 per cent calcium chloride, the lengthening in ten seconds was only 76.17 per cent; in twenty seconds, 92.11 per cent.

Relaxation in sugar takes place at a still slower rate, the amount varying according to the previous treatment which the muscle has undergone. If, for instance, a sartorius is repeatedly immersed in the following solutions in the order given, — K, sugar, Ca, sugar, K, sugar, Na, sugar, etc., — it will be found that the relaxation of a potassium contraction is altered regularly as a calcium or a sodium bath precedes the immersion into potassium. When the immersion into potassium has been preceded by a bath in calcium chloride, the relaxation in sugar equals 57.32 per cent of the total amount in ten seconds; 84.50 per cent in twenty seconds. When a bath in sodium chloride has preceded the potassium contraction, then the relaxation in cane-sugar equals 40.88 per cent in ten seconds; 74.12 per cent in twenty seconds. The effect of the calcium bath is, therefore, not removed by washing for an equal length of time in cane-sugar, and

similar changes can be detected in the height of the contractions and in the latent periods. The potassium contractions preceded by a calcium bath are of less height (about 6 per cent), while the latent period is about doubled. The effect of the calcium chloride, supplied as it is in great amount, is not favorable to the potassium contractions, —a result directly the reverse of that reached for small amounts. It should be stated, however, that the quicker relaxation in cane-sugar after a previous bath in calcium in contrast to a previous bath in sodium does not hold true if the muscle instead of being transferred to sugar remains in the potassium chloride. Under these conditions the relaxation, after a previous bath in calcium, is indefinitely prolonged.

Similarly it can be shown that preliminary treatment of the muscle with cane-sugar solutions for varying lengths of time just previous to immersions into potassium chloride alters the results. The latent period is lengthened and the height of the contraction is diminished. Preliminary treatment with potassium chloride produces the same results more rapidly. If a muscle is made inactive to potassium by alternately placing in potassium and sodium, it may, when treated to long baths in 0.7 per cent saline, show considerable recovery. In one such experiment in which the sartorius was subjected to sodium chloride for two, five, eight, ten, twenty, and forty minutes, it was found that the ability of the muscle to contract was restored at the end of five minutes, but the contraction was barely visible. The power of the muscle to shorten increased with the duration of the immersion in sodium chloride, until at the end of forty minutes the contraction reached $66\frac{2}{3}$ per cent of the height of the original contraction. Twenty seconds' sojourn in potassium chloride again destroyed the power of the muscle to contract. A Ringer solution under similar conditions can restore the muscle within one minute.

Based on the theory of the diffusion of ions into and out of the muscle, the above experiments may be explained as follows: Potassium produces a contraction followed by a relaxation in the sartorius when the two kations, sodium and calcium, are present in physiological amounts. If potassium and sodium solutions alternate so that the calcium present in available form diffuses out, then potassium rapidly becomes ineffective. If potassium and calcium solutions alternate so that sodium ions diffuse out, then potassium also becomes ineffective in producing further contractions, for the muscle enters into a state of rigor. It has been shown by many observers

that if a muscle is placed in potassium chloride the irritability, contractility, and conductivity rapidly disappear. Within certain limits washing with sodium chloride, calcium chloride, Ringer, or sugar solutions will re-establish contractility to varying degrees. After longer exposure this is impossible. Washing with Ringer supplies immediately the kations calcium and sodium. Alternating potassium with cane-sugar permits potassium contractions only during the time required for the ions of the muscle to diffuse out, and sugar has no power of restoring potassium contractions. Subjection of the muscle to calcium chloride produces definite changes that cannot be obviated by mere bathing in cane-sugar solutions for the same length of time. These changes manifest themselves, upon applying potassium, in an enormously prolonged contraction. Neither can a bath in 0.7 per cent saline obviate the changes produced by calcium. A muscle which has lost its power of responding to potassium by being alternately immersed into potassium and sodium will within one minute again respond vigorously if so minute a quantity of calcium as is present in a Ringer solution be supplied.

From a consideration of the phenomena exhibited by the sartorius when immersed in isotonic solutions it seems that the reactions of the muscle are influenced in two ways: first, by the mere fact that one solution is diluted by the presence of another, which thus delays the typical effects; second, the solutions in question have specific effects upon the contractile tissue, which may be alike or may be antagonistic. It can readily be inferred that the concentration of the potassium chloride solutions exercises an effect upon the contraction called forth. In one experiment the sartorius was submitted alternately to a 1 per cent solution of potassium chloride and Ringer until a series of contractions was well established. The employment of a 0.5 per cent solution instead of the 1 per cent effected a diminution of 55 per cent in the shortening. Change to a 10 per cent solution produced a contraction higher than any other of the series, but the deleterious effects were equally marked. When potassium and sodium or potassium and calcium are mixed in various proportions, but in such a way that the mixture always contains 1 per cent of potassium, and these mixtures are applied alternately with Ringer to the sartorius, they rapidly destroy the irritability of the preparation. The thirty-six tests thus made indicate that calcium is somewhat more effective than sodium in preventing a potassium contraction in a fresh muscle, but that when potassium is at all effective the mixture

of potassium plus calcium favors prolongation of the contracted state.

The rapid contraction and relaxation of the frog's sartorius in response to a 1 per cent solution of potassium chloride offers an interesting problem. It can very readily be shown that the normal living condition of the muscle is essential, for anæsthesia produced by ether vapor destroys the potassium contraction as quickly as it destroys the ability of the muscle to respond to electrical excitation. Furthermore, exhaustion of the muscle in 0.7 per cent saline by repeated electrical stimulation prevents the action of the potassium. Only a few experiments were made in this respect, but they indicated that the diminution in the height of the potassium contraction varied with the amount of fatigue of the muscle. This is only an extension of the results of experiments detailed above in which it was shown that previous exposure of the muscle to potassium, calcium, sodium, or sugar altered the normal condition, so that the response to potassium was modified. These substances are injurious to the muscle either *per se* or indirectly by allowing diffusible constituents to leave the muscle, or they are injurious in both ways. In the order of their injuriousness, if supplied alone, they seem to stand,—potassium chloride, calcium chloride, and sodium chloride. It requires but a short sojourn in potassium chloride, a longer one in calcium chloride, and a relatively very long exposure to sodium chloride to destroy the effectiveness of subsequent applications of potassium. Stimulation of the muscle with break-induced currents while submerged in these solutions shows the same order in the disappearance of excitability.

It seems obvious, therefore, that a 1 per cent solution of potassium chloride can produce its characteristic effects only when the muscle is in normal condition. There is a good deal of evidence that the maintenance of the latter is dependent, among many other conditions, upon the presence of the ions sodium and calcium, and the difference in the results obtained by alternating potassium and sodium, on the one hand, and potassium and calcium, on the other, is very significant. In the alternation of potassium chloride and sodium chloride the calcium ions diffuse out of the muscle, and it is conceivable that the loss of contractility is proportional to the loss of calcium ions. When calcium chloride and potassium chloride are alternated, sodium ions leave the muscle, which then promptly goes into a state resembling contracture.

These facts seem to point to the conclusion that potassium pro-

duces a contraction by an interaction involving calcium, and not, as is implied in the publications of Zoethout,[1] by the combined effects of an increase in the potassium content and a removal of the inhibitory influence of calcium. Furthermore, if the calcium present in the muscle inhibits the tendency of the sodium to produce a shortening, and if furthermore the access of potassium by antagonizing the calcium unmasks the tendency of sodium to produce a shortening and so induces the "potassium contraction," then it is to be expected that an alternation of potassium and sodium would call out a long series of contractions or at least a condition of contracture. But this is not the case. If, on the other hand, it is the calcium which is especially involved in the potassium contraction, then an alternation of potassium chloride and calcium chloride should bring out a series of contractions and tendency to contracture, which as a matter of fact actually occurs. Moreover, when after an alternation of potassium chloride and sodium chloride the potassium is no longer able to evoke contractions of the muscle, a supply of calcium restores to its full extent the power of the muscle to respond.

Additional evidence of the importance of calcium to the muscle is the fact that the application of reagents that are precipitants of calcium, like sodium sulphate or sodium oxalate, destroys the ability of the potassium solution to produce contractions. A mixture of 0.7 per cent saline and a saturated solution of sodium oxalate in proper proportions induces contractions which to all appearances are like those produced by potassium chloride. It is very tempting to assume that the method of action of both is the same. Martin[2] in his investigation on terrapin heart strips was led to the hypothesis that there is a spontaneous conversion of calcium from an ineffective to an effective form, particularly under the influence of sodium chloride. That a similar conversion may take place in skeletal muscle is made probable by immersing a sartorius alternately into potassium chloride and sodium chloride until potassium no longer produces contractions. If the muscle is then allowed to remain in 0.7 per cent saline for ten or fifteen minutes, it is found that the muscle has regained to some extent its power of responding to potassium. It is not inconceivable that when potassium is suddenly supplied to the muscle in great quantity it may, in common with the oxalate acceler-

[1] Zoethout: This journal, 1902, vii, p. 320.
[2] Martin: This journal, 1904, xi, p. 103.

ate, the conversion of calcium from an ineffective to an effective form and thus produce a sudden contraction.

Potassium chloride stimulates the muscle, but not in the ordinary way, for the contractions produced are not propagated along the length of the fibre. This is shown in the relations between the height of the contractions produced and the extent of the immersion. Table II gives the results of a typical experiment. The extent of immersion was only approximately that of the figures given.

TABLE II.

The relation of the height of contraction to the extent of immersion. The sartorius muscle of the frog alternately immersed to varying extent in 1 per cent potassium chloride and a Ringer solution. Magnification 3 ×.

Extent of immersion.	Height of contraction.	Calculated height.
	mm.	mm.
About $\frac{3}{4}$	$21\frac{1}{2}$	24
About $\frac{1}{3}$	9	8
About $\frac{1}{3}$	13	$10\frac{2}{3}$
About $\frac{3}{4}$	28	24
$\frac{1}{1}$	32	

If a sartorius is fastened at its centre in such a way as to hold it firmly but so as not to prevent the passage of the wave of contraction called out by electrical or mechanical stimuli, and the free ends of the muscle are connected with recording levers, it will be found that only that half of the muscle contracts that is subjected to the potassium. If the one end of a fresh muscle under such conditions has been injured so that quickly plunging into 0.7 per cent saline will close the demarcation current, it will be found that the impulse thus originated sweeps over the entire muscle.

It has already been stated that the potassium contraction fails in fresh strips of the terrapin heart. Both cardiac and skeletal muscle show greater sensitiveness to the action of salts after exposure to conditions which are not strictly normal. The contraction which results, therefore, after special treatment of heart strips may be analogous to the contraction in striated muscle, but in its various phases it is much more sluggish.

The Fibrillary Twitches in Sodium.

Since 1881, when Biedermann[1] first reported that immersion of striated muscle in an alkaline solution of sodium salts calls forth fibrillary twitches, this fact has been verified repeatedly. In the experiments upon which this paper is based these characteristic sodium twitches were produced in practically 100 per cent of the number of sartorii placed directly into saline. The power of calcium chloride and of potassium chloride to inhibit the twitches was fully corroborated, both when small and when large additions were made to the solution. With minute doses the calcium acted rather more promptly than the potassium. It is stated that the fibrillary twitches may continue for days at a sufficiently low temperature. Skeletal muscle differs in this respect from heart strips of the terrapin's ventricle, which according to Martin reach sodium chloride exhaustion in about one hour and fifty minutes. Both the twitches of the sartorius and the beats of the heart strips are promptly inhibited by large doses of potassium or calcium, for example, by a 1 per cent solution. But here again a difference is to be noted. In minute doses the sartorius twitches are indeed inhibited by calcium or potassium, and the latter may also inhibit the heart, but calcium in suitable amounts improves strikingly the beat of the heart. The effect of diluting with cane-sugar the saline solutions into which the heart strips are immersed has been described by Howell.[2] The behavior in this case is similar to that observed in the muscle. The twitches of skeletal muscle and the beats of the heart strips do not begin promptly, but have a latent period, which is very short and often absent in the sartorius, but according to Martin averages forty minutes in the terrapin heart.

The results obtained by partial immersion of the sartorius into 1 per cent solutions of potassium chloride led to a repetition of the experiments with saline solutions. The simplest way in which this can be done is to place the muscle on the edge of a glass slide in such a way that the vertical half hangs down into 0.7 per cent saline, while the horizontal half rests on the slide in a pool of Ringer's solution. In these experiments the twitches were confined for varying lengths of time to that portion immersed in the saline, before the more vigor-

[1] Biedermann: Electrophysiologie, i, p. 113. Translated by Welby.

[2] Howell: This journal, 1902, vi, p. 181.

ous twitches spread to or involved the half immersed in the Ringer. This experiment was carried over to strips taken from the terrapin's ventricle with similar results. The method of the experiment, however, was slightly altered. Two beakers were placed side by side, one filled to the brim with a Ringer solution and the other with 0.7 per cent saline. Long strips from the ventricle were hung over the adjoining edges of the beakers so that each half of the strip dipped into one of the solutions. Under these conditions the beats began in the end in sodium chloride, and for a short time were limited to this end. As they grew in intensity they involved more and more of the portion of the strip in the Ringer solution until the entire strip was active, — the sodium chloride end, however, initiating the beat. The period of transition from partial to total activity of the strip was often marked by an independence of the rate of the beat of the two ends, so that the sodium chloride end beat three or two times to each beat of the end in the Ringer.

The Contraction Produced by Calcium.

Calcium in percentages above normal in solutions of sodium chloride or Ringer produces marked increase in the tone of both sartorius muscle and ventricular heart strips.

The slow contraction passing into an equally slow relaxation when the tissues in question are placed in 1 per cent solutions is accelerated in both by previous immersion into 8 per cent cane-sugar. That the calcium contraction is localized to the portion immersed in the solution can be demonstrated as in the case with potassium chloride. The following experiment was performed. A strip from the terrapin heart was lightly tied at the middle and fastened immovably to a glass standard. The free ends of the strips were connected with recording levers. One end was then subjected to 0.7 per cent sodium chloride; the other end to 1 per cent calcium chloride. The latter had just completed the slow contraction and relaxation resulting from the immersion into calcium chloride when the beats set up in the saline end began to pass the ligature. The curve traced on the drum by the calcium end of the muscle now showed a marked increase in tone, and exhibited irregular waves formed by groups of contractions. The saline end of the strip showed only progressive loss of tone.

THE BEHAVIOR OF THE SARTORIUS WHEN STIMULATED PERIODICALLY.

If the sartorius muscle of the frog while immersed in a 1 per cent solution of potassium chloride is stimulated once per minute with maximum break-induction shocks, it manifests a rapidly decreasing series of contractions. It is convenient in this and all similar experiments to permit the solution in question to bathe the muscle for fifty-five seconds in every minute. The remaining five seconds give ample time to remove the beaker containing the solution, to give the stimulus, and replace the solution. A series thus obtained consists of contractions varying from one to ten in number, progressively declining in height. After each contraction there is complete relaxation to the base line. The series of contractions becomes longer when the potassium solution is diluted with 0.7 per cent saline, and grows in length as the latter forms more and more of the mixture.

If the sartorius in a similar manner is submitted to a 1 per cent solution of calcium chloride, the resulting curve is quite different. In one experiment the height of the contractions diminished from forty millimetres to three millimetres during the first nine minutes. The diminution thereafter was much slower, so that in three hours the contractions were still one millimetre high. In the mean while certain tone changes had taken place which raised the base line to a height of twenty-four millimetres in one and one-half hours. The tone was then again lessened for an hour and a half, when the original level was reached. The entire curve therefore consisted of nine initial contractions, rapidly diminishing in height, followed by a series of small contractions superimposed upon a tone curve of three hours' duration. The superimposed contractions were executed rapidly with equally rapid relaxations, which gave the impression of an internal support. In describing the tone changes following immersion into a 1 per cent solution of potassium chloride it was found convenient, in order to explain the phenomena, to assume the presence of two contractile elements. A similar assumption can be made in the case of calcium chloride where the rise in tone can be regarded as due to the reaction of the more sluggish element.

Stimulation of the sartorius immersed in a 0.7 per cent saline solution gives a decidedly longer series of contractions than when the immersion is into potassium chloride or calcium chloride, but not so good a series as in a Ringer solution. Sodium chloride, though less

injurious than the potassium or calcium chloride, nevertheless has an effect. It leads to a state resembling sodium chloride exhaustion in heart strips. When in this condition physiological amounts of calcium chloride restore, sometimes to a remarkable degree, the vigor of the contractions, and the action of calcium in this respect stands in contrast to the inhibitory effect it possesses on fibrillary twitches. In Fig. 2 this difference is illustrated. The same amount of calcium applied after the same length of time stops the twitches in an unstimulated sartorius, while it restores the contractions of the stimulated muscle.

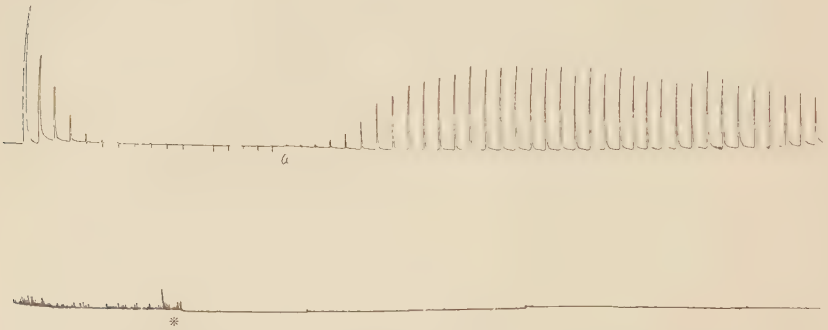


FIGURE 2. — Upper curve. — The sartorius muscle of the frog alternately stimulated by tetanic stimuli of fifteen seconds' duration and then immersed into 0.7 per cent NaCl for forty-five seconds. At (*a*) 0.072 per cent $CaCl_2$ added to the saline. Lower curve. — Sartorius muscle of frog immersed in 0.7 per cent NaCl, but not stimulated. At * 0.072 per cent $CaCl_2$ added to the saline.

This apparent discrepancy finds an explanation in the assumption once more of two contractile elements in the muscle. One of the elements on this supposition was found to be very sensitive to potassium chloride; the other just the reverse, so that it reached a maximum contraction only in twenty-four hours. Calcium chloride as a 1 per cent solution, on the other hand, was found to diminish the contractions of the first contractile element, and its action on the second was much more rapid than that of potassium, producing a maximum contraction in one and one-half hours. If sodium occupies a position between potassium and calcium in its ability to produce its effect upon the two contractile elements, then the salts in the order of their efficiency upon the first element would stand potassium, sodium, calcium. In their efficiency of producing an effect upon the second element they would stand in the reverse order, — calcium, sodium, potassium. If the twitches of the sartorius are due to the action of

the sodium salt on the first contractile element, then the addition of calcium would prevent them, since calcium inhibits the activity of this element. After the preliminary potassium-contraction potassium inhibits the activity of muscle tissue so that the addition of this salt would also prevent the twitches. Weakened contractions resulting from periodical electrical stimulation in saline and involving both contractile elements might be augmented by the addition of calcium, but since one element is inhibited and the other favored, variations in the extent of the recovery might be expected, and this in fact may be noticed. In some cases the recovery may be considerable, as in Fig. 2, while in other cases the effect produced is hardly more than a retardation of the decrease in height of the individual contractions.

To the two hypothetical contractile elements of the sartorius can be added a third, present in the ventricular muscle of the terrapin heart, which manifests a still greater sluggishness to the action of potassium chloride, responds with more vigorous contractions to immersion into sodium chloride, and is by far more affected by calcium chloride. In such a scheme the second contractile element of striated muscle is more nearly similar in its reactions to cardiac muscle than is the first, but the differences and similarities between the three are not absolute, but rather a matter of degree. Dr. Howell has called the author's attention to the difference between the behavior of terrapin's striated muscle and cardiac muscle when subjected to chloroform vapor of a certain degree of density. The striated muscle will contract vigorously, but the heart muscle relaxes with almost equal rapidity. Nevertheless, if the chloroform is made to act with greater rapidity and density, both kinds of tissue shorten.

With regard to the action of the ions of potassium, sodium, and calcium, the idea of the differences between skeletal and cardiac tissue being one of degree can be maintained on the assumption that the first and second contractile elements of the sartorius and the contractile element of the heart form a series of different sensitiveness to the ions in question. Thus the first contractile element of the sartorius is readily affected by the potassium ions, the second contractile element less so, and that of heart tissue not at all unless subjected to special treatment such as previous immersion into cane-sugar. Again, under the influence of sodium chloride the first contractile element responds by twitching, the second element possibly gives rise to larger and less numerous contractions, and heart tissue responds with well-developed co-ordinated contractions. That the second contract-

ile element of the sartorius may respond to immersion into sodium chloride is suggested not only by experimental results, but by the statements of Biedermann and Loeb,[1] who have described an alternation of minute twitches and larger contractions when striated muscle is placed in sodium solutions. Zenneck[2] has described the behavior of the curarized sartorius in $\frac{n}{10}$ sodium bromide solutions as consisting of twitches grouped together so as to appear like blood-pressure curves showing respiratory waves. Lastly, the first contractile element of the sartorius is not at all or very little stimulated by calcium; the second contractile element more so, and heart tissue most of all.

It is impossible to state to what the differences in the reactions of the three contractile substances towards the chlorides of potassium, sodium, and calcium are due. But it is suggested by the experiments with pure solutions that the ion content of the muscle is of great importance. The power of potassium to produce a shortening is impossible when only potassium and sodium are present in the muscle. A physiological percentage of calcium favors the potassium contraction, but when the amount is greatly increased it markedly retards the same reaction. Calcium when applied to a tissue in which the ions are present in physiological amounts leads generally to a shortening, but when applied at the height of a potassium contraction leads to rapid relaxation. Considerations of this kind are also of service in forming a conception of the reasons underlying the less constant variations which muscle exhibits.

As early as 1887 Ringer[3] and Ringer and Buxton[4] undertook a comparison of the effects of sodium, calcium, and potassium solutions upon skeletal and cardiac muscle. They did indeed find both differences and similarities, but certain conclusions in the two papers seem radically opposed. These are attributed to differences in the method of application of the solutions, — immersion in one case and perfusion in the other. Thus upon immersion and periodical stimulation of the sartorius Ringer found that skeletal muscle differed from cardiac muscle in the following particulars: "Contractility is not improved or restored by adding lime salts to saline." "The contractions in saline of a muscle previously weakened by frequent contrac-

[1] Loeb: Ueber Ionen welche rhythmische Zuckungen der Skelettmuskeln bevorrufen, Festschrift für A. Fick, Braunschweig, 1899.

[2] Zenneck: Archiv für die gesammte Physiologie, 1899, lxxvi, p. 21.

[3] Ringer: Journal of physiology, 1887, viii, p. 20.

[4] Ringer and Buxton: Journal of physiology, 1887, viii, p. 288.

tions are strengthened by adding to saline a potash salt." In the later paper by Ringer and Buxton the experiments were performed by perfusing the gastrocnemius with a 0.6 per cent saline solution and stimulating periodically. It was found that excitability had about ceased in an hour and three-quarters. "Upon replacing saline by phosphate of calcium saline a return of contractility took place." "We then returned to saline, and again contractility disappeared, to be restored by returning to the phosphate of calcium saline; and this manœuvre was effected several times, always with the same results." In the experiments underlying this paper in which the frog's sartorius was simply immersed in the solutions and stimulated periodically, potassium was never observed to favor contraction, — its effects being rather the reverse. Calcium, however, after sodium chloride exhaustion almost invariably increased the height of the contractions to some extent, although as stated by Ringer the extent to which recovery occurs varies greatly in different experiments. The experiments in Ringer's earlier paper were performed during the months of May and June, when frog preparations are liable to manifest abnormalities of behavior.

As far as direct experimental evidence goes it would seem that the sartorius is characterized by responding with a rapid contraction when submitted to a 1 per cent solution of potassium chloride, — a result which does not occur in heart tissue when in a fresh condition, — and that this forms a point of difference between the two tissues. Another difference is the reaction to physiological amounts of calcium chloride after treatment with 0.7 per cent saline. Calcium under these conditions inhibits the activity of the sartorius while it augments the activity of heart tissue. But these dissimilarities cannot be looked upon as the expression of sharply marked differences of structure of the two tissues, since treatment with cane-sugar makes ventricular tissue responsive to potassium, and periodic stimulation of the sartorius while in saline changes the inhibitory effect of calcium to an augmentor effect. The latter phenomenon finds an explanation in much better accordance with other known facts by the assumption of two contractile elements in the sartorius, which are differently affected by calcium. On this basis the fibrillary twitches of the sartorius and the rhythmical beats of heart tissue would not be strictly analogous, since they are, to a large extent at least, the manifestations of contractile substances with different properties.

Conclusions.

1. That a supply of the ions sodium, calcium, and potassium in definite proportions is necessary to the best maintenance of the normal activity of skeletal muscle of the frog and heart muscle of the terrapin.

2. That in physiological proportions sodium and potassium produce a condition of relaxation, while calcium produces a condition tending toward contraction, provided all three ions are present in the medium which bathes the muscle. This is true for both heart and skeletal muscle.

3. That the effects of the three ions as stated in 2 are not necessarily the same when the ions are supplied separately to fresh tissue. In isotonic solutions sodium produces a relaxation in both heart and skeletal muscle. Calcium produces a slow contraction in both. Potassium produces two contractions and relaxations in the sartorius, but only relaxation in a fresh strip of the terrapin's ventricle.

4. That the condition of the tissue influences the effect produced by any one ion. Thus previous treatment with sodium chloride, calcium chloride, potassium chloride, or cane-sugar solutions alters the condition of the tissue so that its reactions are modified.

5. That the effect of any one ion may be modified by the simultaneous application of other ions.

6. That the rapid contraction in skeletal muscle produced by a 1 per cent potassium solution and the slow contraction resulting from immersion into a calcium solution are restricted to the portions immersed.

7. That the sodium twitches in skeletal muscle and the rhythmical contractions in heart muscle are at first limited to the immersed parts, but soon spread to those portions not in the solution.

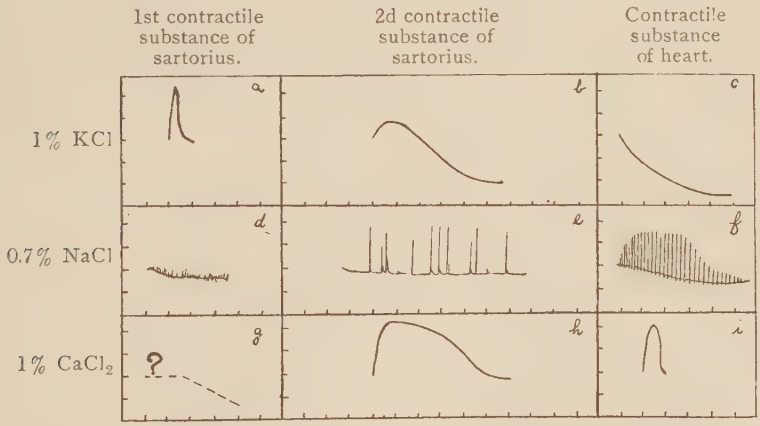
8. That the contraction and relaxation produced by a potassium solution when applied to the sartorius will not occur unless both sodium and calcium are present in the tissue.

9. That the power of potassium chloride to produce a contraction is antagonized by the presence of either sodium or calcium in the bathing medium, but the antagonism of calcium is the more marked.

10. That the effects produced by an alternation of potassium and sodium, on the one hand, and of potassium and calcium, on the other, lend support to the view that the calcium ion is more intimately associated with the contraction process than is the sodium ion.

11. The assumption is made that there are two contractile substances in the sartorius muscle and a third in the terrapin's ventricle. No suggestion is made as to the relation of these substances to definite histological elements, as in the views proposed by Grützner and by Bottazzi. Evidence, however, is offered that the contractile substance of the heart in its properties bears a closer resemblance to the second than to the first contractile substance of skeletal muscle. These substances differ in their sensitiveness to the action of sodium, potassium, and calcium chloride. The first contractile substance of the sartorius responds quickly with a contraction when subjected to a 1 per cent solution of potassium chloride; the second contractile element responds much more sluggishly, and ventricular muscle when in a fresh state gives only relaxation. Calcium chloride in a 1 per cent solution produces no contraction of the first contractile element of the sartorius, gives rise to a slow contraction of the second contractile element, and produces quite a vigorous contraction of heart muscle. Immersion into a 0.7 per cent solution of sodium chloride is followed by fibrillary twitches of the first contractile element of the sartorius; by larger but infrequent contractions of the second contractile element, and by well-developed rhythmical beats in heart strips.

The following diagrams may serve to illustrate these differences. No significance can be attached to the exact form of the curves, but the time relations in a general way are reproduced.



Each division along the ordinates represents a change in the length of the muscle of six millimetres. Each division along the abscissæ is equal in (*a*) to one minute; in (*b*) to one day; in (*c*) to one day; in (*d*), (*e*), and (*f*) to fifteen minutes; in (*h*) to one day; and in (*i*) to one hour. (*g*) represents a supposed relaxation of the first contractile substance in 1 per cent $CaCl_2$.

This assumption offers an explanation of the different effects of small amounts of calcium upon the fibrillary twitches of the sartorius on the one hand, and upon the contractions of the muscle when periodically stimulated, on the other hand. Calcium stops the twitches because it inhibits the activity of the first contractile element which produces the twitches. The contractions of the sartorius when stimulated electrically involve the second contractile substance as well as the first, and calcium in suitable amounts favors the activity of this element. Upon this assumption, furthermore, the fibrillary twitches of the sartorius in sodium chloride are not strictly analogous to the beats of heart tissue in sodium chloride, since they involve the activity of different substances.

BIOGRAPHICAL SKETCH.

August Ernest Guenther was born in Sandusky, Ohio, January 20th, 1874. He attended the public schools of his native city until his 18th year, when he entered the academic department of the University of Michigan. He served as assistant to the curator of the university museum from 1894 to 1897. From 1897 to 1901 he acted as assistant in physiology in the medical department of the University of Michigan. He received the degree of S. B. in biology in 1898. Entered the Johns Hopkins University as a graduate student in 1902, pursuing studies in physics, anatomy and physiology. He held the fellowship in physiology during the year 1903-'04, and was made assistant in physiology in 1904.

Johns Hopkins University,
Baltimore, Maryland, May, 1905.